Supplemental Data

The Inability to Disrupt the Immunological Synapse distinguishes HIV-1 from most other Primate Lentiviruses

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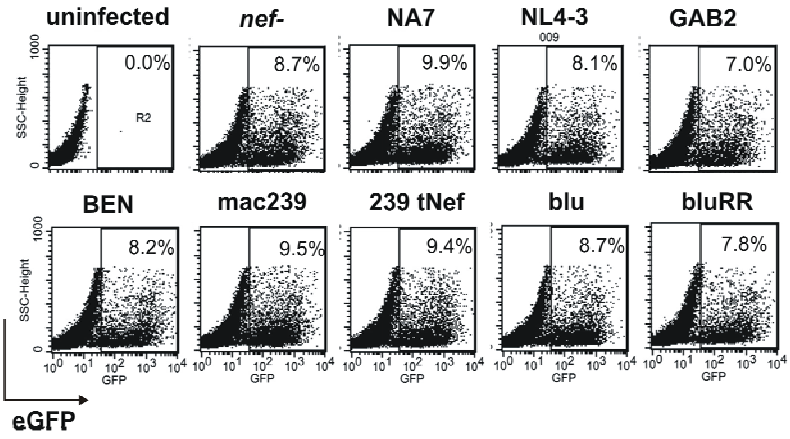
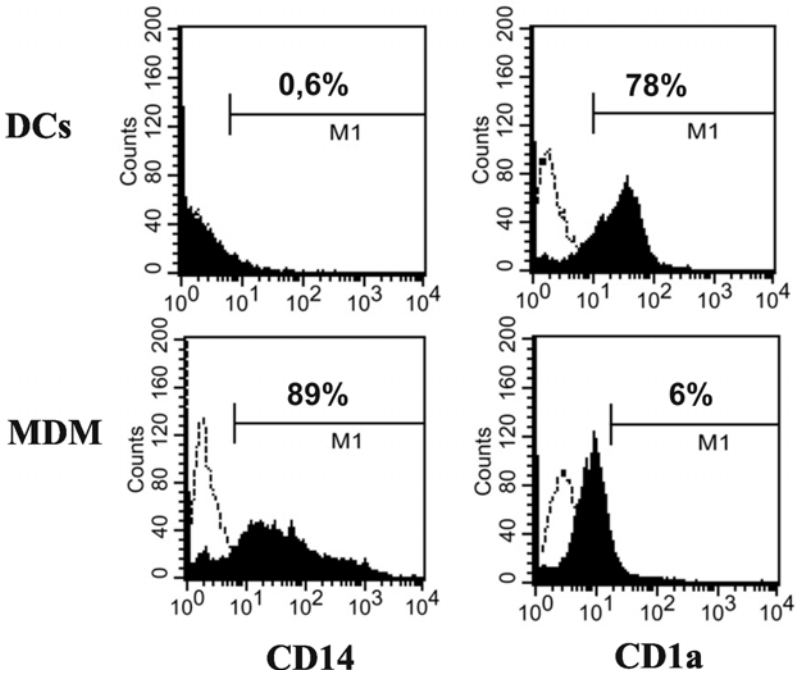
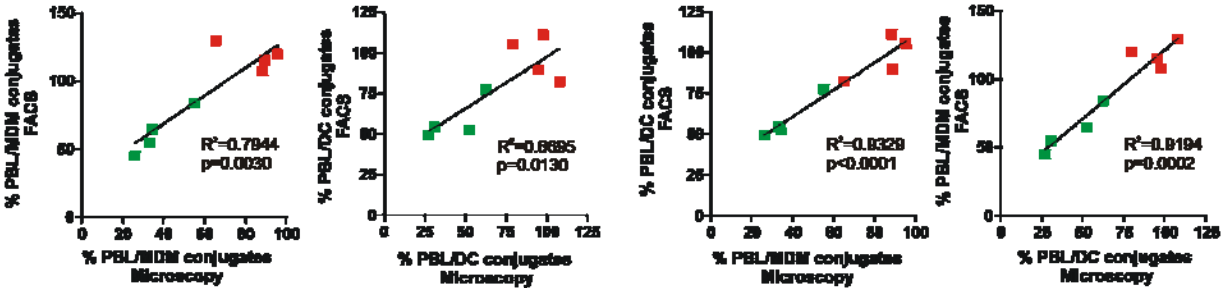
Supplementary Figure 1: Quantification of HIV-1 infection efficiencies. PBLs were infected with VSV-G pseudotyped HIV-1 particles containing normalized quantities of p24 (50 ng) and analyzed by flow cytometry 3d later. The numbers give the percentages of eGFP$^+$ (HIV-1-infected) PBLs of the total number of cells analyzed.

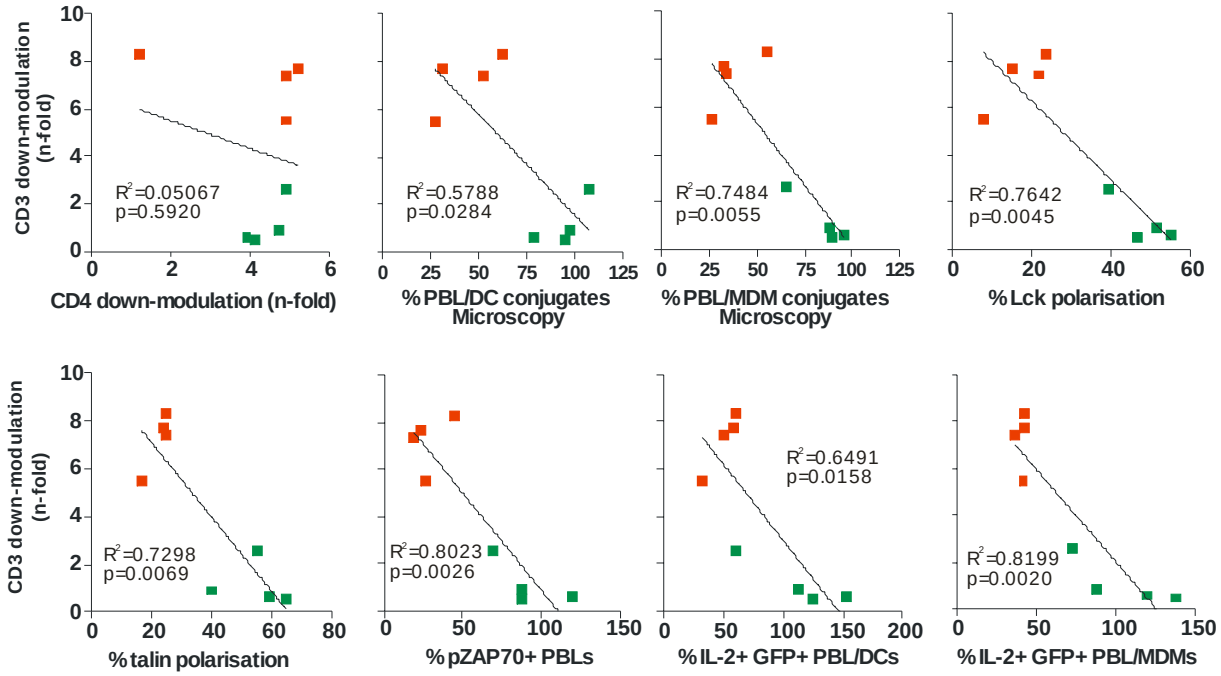


Supplementary Figure 2: Phenotypic characterization of primary DCs and MDMs. Flow cytometry analysis was performed on monocyte-derived DCs and MDMs at 5 and 7d post-differentiation, respectively. Dotted lines show isotype controls.

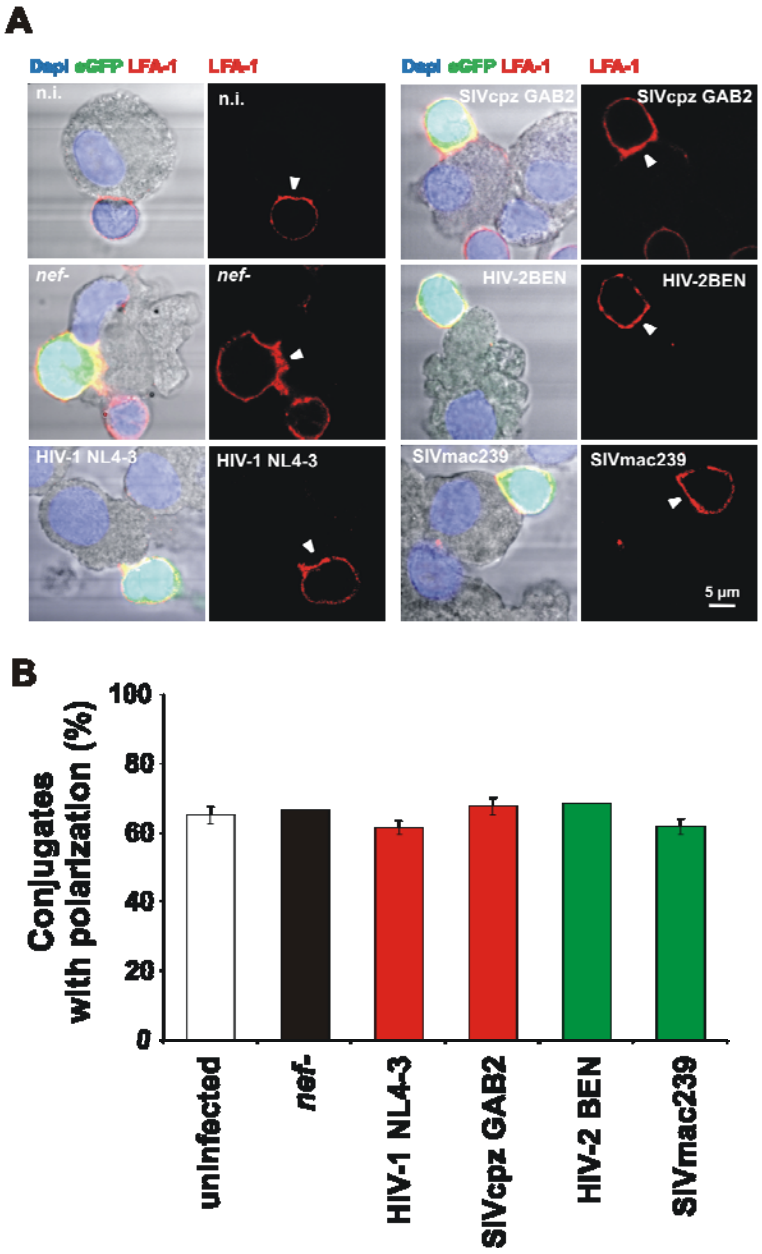




Supplementary Figure 3: Correlation of results from microscopy examination (Figure 1A) and flow cytometry analyses (Figure 1B) to assess complex formation between infected PBLs and autologous MDMs or DCs. The values used for the calculations are summarized in Table S2 and give the percentages of HIV-1-infected PBLs that formed complexes with autologous SEE-pulsed MDMs or DCs relative to those infected with the *nef*-defective control virus (100%) in the graphs. Results obtained using viral constructs expressing *nef* alleles that downmodulate TCR-CD3 are indicated by green and those that do not by red symbols.



Supplementary Figure 4: Correlation between the efficiency of Nef-mediated down-modulation of TCR-CD3 and the modulation of CD4 cell surface expression, the formation of conjugates between virally infected PBLs and DCs or MDMs, the polarization of Lck and talin towards the contact zone, ZAP70 phosphorylation and IL-2 induction. All values used for linear regression analysis are provided in Table S2.



Supplementary Figure 5: Nef does not impair polarization of LFA-1 at the immune synapse. (A) $CD4^+$ T cells infected with HIV-1 constructs expressing the indicated *nef* alleles were incubated with autologous sAg-pulsed DCs, fixed and stained with anti-LFA-1. The right panels show LFA-1 antibody labeling and left panels a merge of eGFP, Dapi and LFA-1. (B) Number of HIV-1 infected $eGFP^+$ T cells engaged in complex formation with APCs showing a polarization of LFA-1 at the zone of contact. The graphs show the mean of two independent experiments +/- standard error of the mean, except for HIV-2 BEN which represents a single experiment.

Table S1. Modulation of cell surface receptors by HIV and SIV *nef* alleles

		GenBank		down-modulation (n-fold) of:				
Lineage	Clone	Accession #	Species/subspecies	CD4	MHC-I	CD3	CD28	Group
HIV-1/M	NA7	DQ242535	Human (*Homo sapiens*)	7.2	10.4	1.0	5.4	1
HIV-1/M	NL4-3	M19921	Human (*Homo sapiens*)	6.6	12.1	1.0	2.6	1
SIVcpz*Ptt*	GAB2	AF382828	Central chimpanzee (*Pan t. troglodytes*)	7.3	8.6	0.8	5.8	1
HIV-2	BEN	M30502	Human (*Homo sapiens*)	4.8	5.9	16.7	7.9	2
SIVmac	239	M33262	Rhesus macaque (*Maccaca mulatta*)	7.0	9.1	16.4	16.9	2
SIVmac	239 tNef	n.a.	n.a.	1.2	1.13	12.4	1.9	2
SIVblu	KE31	DQ222474	Blue monkey (*Cercopithecus mitis*)	6.9	11.0	18.0	20.0	2
SIVblu	RR-AA	n.a.	n.a.	5.8	7.3	1.8	14.3	2

The Nef-mediated *n*-fold down-modulation of CD4, MHC-I, TCR-CD3 and CD28 on HIV-1-infected PBMCs was calculated as the mean fluorescence intensity (MFI) in cells infected with HIV-1 constructs coexpressing Nef and eGFP relative to cells infected with a *nef*-deficient HIV-1. The results were derived from Ref. 30. Group 1 and 2 Nefs indicate Nefs that do not and do down-regulate TCR-CD3, respectively.

Table S2. Effect of HIV and SIV *nef* alleles on the interaction between virally infected T cells and APCs

		modulation of[1]		Infected PBLs interacting with[2]				polarisation of [3]			PBLs staining positive for[4]		
Lineage	Clone	CD3	CD4	DC-F	MDM-F	DC-M	MDM-M	Lck	Talin	LFA-1	Zap70$^+$	IL-2 DCs	IL-2 MDM
HIV-1/M	NA7	0,6	3,9	88,4	97,7	107,8	111,3	51,3	40,0	n.d.	87,0	112,0	87,4
HIV-1/M	NL4-3	0,9	4,7	95,4	79,4	120,0	105,7	55,0	59,3	61,3	120,0	153,4	119,8
SIVcpz*Ptt*	GAB2	0,5	4,1	89,0	95,0	115,6	89,8	46,7	64,8	67,4	88,1	124,7	138,7
HIV-2	BEN	5,5	4,9	25,9	26,9	45,0	49,4	7,5	16,7	68,4	27,2	32,8	41,4
SIVmac	239	7,7	5,2	33,2	30,8	55,0	54,4	15,0	24,4	61,7	23,3	58,0	41,6
SIVmac	tNef	8,3	1,2	55,2	62,3	84,2	77,5	23,3	25,0	n.d.	45,2	59,4	41,5
SIVblu	KE31	7,4	4,9	34,1	52,3	64,8	52,7	21,7	25,0	n.d.	19,4	49,3	35,9
SIVblu	RR-Nef	2,6	4,9	65,4	108,0	129,8	82,5	39,5	55,0	n.d.	69,7	59,7	72,8

[1]CD3 and CD4 surface downmodulation in infected primary CD4$^+$ T cells was determined at 2 days post-infection. Numbers give the ratio between the MFI of cells infected with the *nef*- control construct and HIV-1 constructs containing intact *nef* alleles. Shown are average values derived from three independent experiments.

[2-4] The values were derived from Figures 1 ([2]), 3 to 5 ([3]) and 6 or 7 ([4]).

Abbreviations: n.d., not determined; -F, flow cytometry; -M, microscopy.